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EXPERIMENTAL SEARCH FOR THE ORIGIN OF AUTONOMIC NERVE FIBERS IN THE POSTERIOR ROOT OF SPINAL NERVE

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Received for Publicaton Mar. 11, 1963

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CHAP. I. INTRODUCTION

Many works have been appearing on the existence and its properties of autonomic nerve in the spinal meninges from the sides of physiological, pharmacological, and anatomical view. In 1876 STRICHER was suggestive of that the vasodilator nerve of pelvic limb runs inside the sciatic nerve through the posterior root of spinal cord. BAYLISS named this phenomenon "Antidromic Action" due to the fact that vasodilator nerve histologically has its origin in the sensory nerve fiber and this is not concomitant with the physiological, basic theory of KOELLIKER and LANGLEY as to sensory pathway. According to MÜLLER (1924), vasomotor nerve center in spinal cord lies between the 8th cervical region of spinal cord and the 3rd lumbar region of spinal cord and this connects to the sympathetic ganglion through the anterior root. However, the exodic nerve distributed in blood vessels can extend the fibers directly from the ganglion in the neighborhood of ganglia of sympathetic trunk.

In the case of peripheral vessel, the nerve fibers originated in ganglion stretches to mingled spinal nerves and run parallel to the spinal nerves. BAYLISS stated that the vasomotor nerve extend to the sympathetic ganglionated cord through anterior root of spinal nerve located between the 11th thoracic region of spinal cord and 3rd lumbar region of spinal cord, and through grey rami communicantes while LANGLEY insists the locus between the 6th lumbar region of spinal cord and 2nd sacral region of spinal cord, WERZILOFF between the 4th lumbar region of spinal cord and lst sacral region of spinal cord.

Long time ago, LOVÉN stimulated the center portion of the cross sectional area of N. Saphenus, which led him to observe the temporal vasoconstriction under the control of nervous system and soon the vasodilation followed. Also LANGLEY recognized peripheral vasodilation of hindfoot when the 7th lumbar region of spinal cord of cat is electrically stimulated. On the basis of above observation, LANGLEY proposed a hypothesis as "Axon Reflex" reflected by the occurence that the direct peripheral vasodilation can be observable without the centripetal propagation of sensory nerve. ASCROFT (1937), HINSEY, PHILLIPS (1938) interpret that the mechanism of vasodilation through posterior root of spinal root can not be clarified perfectly at the present.

We need time, unless the entity of nerve fiber being aparent and the working mechanism being observed with un-injured animal, to the perfect understanding. They are also in doubt that the ineffectiveness of dissecting the centripedal fibers of limb on the vascular plexus is the indicative of the occurence of some sort of fiber which effects on vasodilation tensely. Referring to the previous investigation, we deduce that the vasodilator nerve and sensory nerves are very closely related, though the problem is left unsolved whether they are exactly the same.

However, assuming that the vasodilator nerve locates in the posterior root of spinal nerve as mentioned above, as if the dissection of abdominal ganglionated cord caused the loss of vasoconstriction effect and consequently expand the blood vessel of pelvic limb. The vasoconstriction of pelvic limb must be brought about after the dissection of posterior root, though this has not yet been proved.

In 1926, OSAWA made a comment, based on his exp. the increase of blood volume immediately after rhizotomy can be rendered to the peripheral vasodilation due to the mechanical stimulation such as dissection, of which BAYLISS et al also suggested.

Their experiments also evidenced that the loss of capacity of vasodilator nerve naturally ends only in the appearance of vasoconstrictor nerve which should be an antagonistic counter-part of the former. It is worthwhile to mention that the above experiment threw a light to know whether the network of vasodilator nerve does exist in the posterior root of spinal nerve which has long been left unknown.

From their histological standpoints, BIDDER and VOLKMANN (1842) came to the recognition that many of the myelinated nerve fibers begins at the boundary region of sympathetic ganglionated cord and directs toward the terminal portion, passing over spinal nerve. On the other hand, GASKELL (1886-1889), proved a existence of unmyelinated nerve fibers running into the posterior root of spinal nerve through grey rami communicantes of dogs sympathetic ganglionated cord. Almost all the fibers in endorachis so far mentioned can no more be recognizable in the locus of the fasciculus of posterior root of spinal nerve. Although RANSON in 1912 observed the presence of the unmyelinated nerve fiber in the fasciculus of posterior root and that this fiber can be traced down to the spinal cord through ganglion of spinal cord and the posterior root, his observation was counteracted by PARSON in 1912, who pointed out that the nerve fiber was likely to be neuroglia instead of unmyelinated nerve fiber. Through MÜLLER, in 1924, offered an evidence on the existence of unmyelinated nerve fiber which runs into the central nervous system, begining at the grey rami communicantes, the histological evidence still lacks whether this fiber find the direction toward the spinal cord through posterior root.

In 1926, YAMAZAKI observed the groups of unmyelinated nerve fibers, each composed of a few or ten units fibers of 0.7-2.0μ in diameter locates among the myelinated nerve bundles of 1.0-8.0μ in diameter. Technically be employed CAJAL's myelin sheath staining on the cross-sectional specimen of nerve fiber by which the myelinated portion reveals yellow region and the unmyelinated dark-brown. As to its distribution, the surrounding area of nerve trunk was most dense. Osmium staining revealed the distribution of the unmyelinated nerve fiber over the whole length of posterior root. He added a statement that this fiber can be visualized from both inside and outside of endorachis to spinal cord, but of this fiber the locus of the nutrition center was left unknown. In 1928, TUNODA predicted the origin of unmyelinated nerve fiber as beging at the boundary sympathetic ganglionated cord, based on his experiment that the rhizotomy at the upper and lower parts of its ganglion and the following observation led to the appearance of degeneration only in the side of the myelinated nerve fiber.

In 1933, NAKANISHI aparently distinguished two types of fiber in both the anterior and posterior roots by their morphological characteristics with respect to their thickness, color and superficies on 1% osmium stained specimen of Bull frog's root of spinal cord. The results indicated that they were characterized by 2.5-4.0μ in diameter,color in light YELLOWISHBROWN or ordinary YELLOWISHBROWN, and slightly swelling tendency which made the distinguishment of myelinated nerve fiber possible from the small size myelinated nerve fiber.

The small size myelinated nerve fiber can be specified by the relatively thin myelin-sheath ACCOMPANINGED by the wavy folds, and was pointed out belonging to autonomic nerve fibers. Nevertheless, question was not solved whether these myelinated nerve fibers in posterior root are of centrifugal, or centripedal nature. According to NAKANISHI's method, SUGIMOTO investigated systematically the state of autonomic nerve fibers in peripheral nerve in terms of its distribution and reached the point of knowing the presence of plenty of autonomic nerve fibers in posterior root.

By the same method, NAKANISHI recognized the existence of the small size myelinated nerve fiber at Ramus cutaneus cruris lateralis of skin, which is of centrifugal nature and seems to control the cutaneous gland or the other cutaneous organs. One should pay attention to the future clarification as to whether the small size myelinated nerve fibers does mingle so-called centripedal autonomic nerve fibers which begins at skin and run into the spinal cord through grey rami communicantes.

Besides, NAKANISHI questioned to the possible existence of nerve fibers morphologically characterized by the sympathetic nerve fibers in 1% Osmiun stained spinal nerve fibers of Bull frog's Nervus Ishiadicus from which the fractions orginated at grey rami communicantes were removed.

In other words, he made a trial to find out directly any trace of the fibers of autonomic nature together with the spinal nerve fibers from the ganglion of spinal cord, but ended in vain. However it has been well-known that in posterior root the fibers with the same magnitude of diameter as that of autonomic nerve fibers included in plenty. In view of Go's report that small size myelinated nerve is of centrifugal property, NAKANISHI (1933) deduced the possibility that the sympathetic nerve fibers strengthening the skeletal muscular contraction and that working antagonistically against contraction branches indifferently from the anterior and posterior roots. Thus he attempted to observe in the posterior root the occurence of the nerve fibers of morphologically similar to the sympathetic nerve fibers found in the anterior root, and if it is in the occurence, to estimate the rate of its content in each root.

Though it has not been thoroughly investigated, for example, in the VI and VII spinal nerve the numbers included in both roots are found to be equal, but this rate may or may not be encountered in the other root of spinal nerve. Go et al reported the occurrence of the plenty of small size parasympathetic myelinated nerve fiber in the posterior root of lumbar spinal nerve which forms the sciatic nerve of dog.

This portion, samely as in the case of bull frog, forms the empty space in account of the origin of spinal autonomic nerve. Namely, this portion does not extend the preganglionic fiber. Go's opinion is not in line with those of NAKANISHI's with bull frog who insisted on the existence of least number of the small size myelinated nerve in the posterior root. Among the results from Go's exp. only statement that NAKANISHI admits is about the knowledge as to small size myelinated nerve fibers remained intact even after the degeneration experiment by means of posterior rhizotomy. Alternatively, the above statement acknowledges us the existence of centrifugal, small size myelinated nerve fiber. Judging from the observation by NAKANISHI with bull frog's posterior root, the reason to allow consistency is due to the presence of about equal number of autonomic nerve fibers to centripedal autonomic nerve fibers, so that two antagonistic types of nerve fibers, one works stimulatory and the other inhibitory, begin independently at anterior and posterior roots. In addition, provided that these fibers are of centripedal property, unexpectedly small number of fibers can be found in 8th and 9th posterior roots. Consequently, it is predicted that the distribution of centripedal autonomic nerve fiber throughout the hind foot is not sufficient at all, sometimes becomes empty.

LANGLEY introduced a new finding from his degeneration experiment that the intermediary cell body which penetrates into the posterior root through the boundary ganglionated cord of sympathetic nerve does not take place at the ganglion but all at spinal ganglion. The Osmium staining of autonomic nerve fibers enabled NAKANISHI to conclude that autonomic nerve fibers are classified to the myelinated nerve fibers rather than the previously accepted unmyelinated fibers.

In 1959, KOTERA et al re-examined and confirmed this staining method to be the useful means for the classification. In 1920 DIAMAREA de Mennato, examination of autonomic nerve fiber by means of polarized light revealed that the all types of autonomic nerve fibers are, myelinated, if not thick at all. The similar findings were reported by MAXIMOW and BLOOM by use of polarizer, by which frequently the apparent unmyelinated nerve fiber turned to be the myelinated one in reality.

In 1955, GASSER, by his first observation of unmyelinated nerve fiber under electron microscope, proposed a new membrane as "Mesaxon". His observation imlpies that axis cylinder is connected with the boundary membrane of SCHWANN cell via Mesaxon and the micro structure of SCHWANN cell of unmyelinated nerve fiber does not differ at all from that of the myelinated.

Referring to his histochemical standpoints, we are allowed to introduce a hypothesis concerning the classification of two types of nerve fibers, namely, choline susceptible and adrenaline susceptible type nerve fibers proposed by OKINAKA in 1960. In detail, his hypothesis stands on the findings that the autonomic nerve can not simply be devided into sympathetic nerve and parasympathetic nerve system, but better be classified according to the above proposal since the so-called parasympathetic and sympathetic nerve systems from past nomenclature often involves the complication. When dealing with choline-susceptible nerve fibers, the actual role of acetylocholine, choline-esterase, and choline-acetylase has been taking into account, similarly, with regard to adrenaline susceptible nerve fiber, the role of noradrenaline. Dopa-decarboxylase and Monoaminoxidase has been under consideration. Referaing spinal ganglion to above line, the cholinesterase activity in spinal ganglion is extremely lower than that in trigeminal ganglion, while monoaminoxidase activity was partly demonstrated in the nucleus of spinal ganglion. These findings are not in line with the old theories in which the condution mechanisms of vasodilator nerve fiber was roughly classified into Inverse Theory of Nerve Conductance and Theory of Parasympathetic Spinal Nerve Existence. From anatomical, histological and histochemical sides of view, concering vasoconstrictor nervous system the detailed route of autonomic nerve network in posterior root of spinal cord has not yet been neatly demonstrated.

By this reason, our experiments were undertaken with the purpose of attempt to search for the origin of autonomic nerve fibers.

CHAP. II. MATERIALS AND METHODS.

Adult dogs weighed 10kg were anesthetized until the moment of their death. Then, as much amount of spinal cord together with anterior and posterior roots were collected after dissection. Each portion was immersed into 20% formaldehyde and fixed for 3 weeks. Immediately after tissue was separated, one portion was stained in 1% Osmium for 20 min.

Then the tissue was transferred into the 1 to 1 mixture of glycerol and water to soften the specimen rather rapidly. The specimen thus prepared were observed under the phase difference microscope.

SECT. I. FOUNDAMENTAL EXPERIMENT WITH DOGS.

PART I. NOTICE ON THE ANATOMICAL ASPECT OF DOGS.

The backbone of dogs includes 7 cervical vertebra, 13 dorsal vertebra, 7 lumbar vertebra, 3 sacral vertebra, 20-23 coccyx as its constituents. With the purpose of observing lumbar spinal nerve, the skin from lumba portion, namely, from lst spinous process of lumbar vertebra down to the lower part of ilic bone were cut through, and lumbar membrane was cross-sectioned at both sides of spinous process of lumbar vertebra. Without any marked injury, M. serratus posterior, M. longissimus dorsi were dissected to expose process. At this moment, it became possible to reach endorachis aftre Laminectomia. Next, the outflow of cerebrospinal fluid is observable when taking off the endorachis. Avoiding the mark of injury on spinal cord and openning as widely the endorachis on both sides, we came to notice the posterior root of spinal cord running into the endorachis from front and rear parts of spinal cord respectively. Anterior and posterior rhizotomy was performed on the spinal cord of endorachis. As mentioned previously, spinal nerve is composed of 8 nerve trunks in cervical portion, 13 in dorsal portion, 7 in lumbar portion, 5-6 in sacral portion. Anterior and posterior rhizotomy were carried out in the region between 2nd and 5th lumbar spinal nerve, so that for the preparation of specimen 3rd and 4th lumbar spinal nerve could be employed. To expose sympathetic ganglionated cord, the vertical cut was given at the center of abdomen. On the both sides of the outer surface of peritoneum, rectus abdominis muscle, transversus abdominis muscle, obliqnus abdominis muscle, and externus abdominis muscle must be removed during the procedure. This removement of muscles make us go deeper passing over the front part of M. psoas major and M. psoas minor which should be dissected also. If we continue to go along the central portion of M. psoas minor in touch with the front surface of basal portion of vertebra. This would enable us to see the sympathetic nerve trunk with aprox. 7 beading nods on that course. In the region of lumber vertebra at the highest and sacral vertebra at the lowest sympathetic nerve trunk was cut out as widely as possible.

PART Ⅱ. ANESTHESIA

Dogs were anesthetized with Nembutal (50mg pentbal-Na 1c.c.). The musclular injection of 2-3c.c. of Nembutal were performed ca. 30min. before the operation to keep the animal under faint anesthesie.

To obtain deep anesthesia, second injection of 2-3c.c. was followed at cutaneous vein in side thigh, just before laminectomy, anterior and posterior rhizotomy, sympathetic ganglionectomy. Dogs became completely free from suffering at 3 hours period after the onset of anesthesia.

PART Ⅲ. PREPARATION OF SPECIMEN WITH REFERENCE TO A VARIETY OF DISSECTION.

(1) A bilateral application of posterior rhizotomy in endorachis

Specimen : a) Posterior root in the region between dissected part and spinal cord.

b) Posterior root from the outside of endorachis (inclusive of spinal ganglion)

(2) A bilateral application of anterior rhizotomy in endorachis

Specimen : a) Posterior root in the region of central nerve side from endorachis.

b) Posterior root in the region between endorachis and ganglion of posterior root of spinal cord.

c) Region right below the spinal ganglion of posterior root (peripheral)

(3) A bilateral application of central rhizotomy on the spinal ganglion.
Specimen : a) Posterior root in the central nerve side from the cut face.

(4) A bilateral application of posterior rhizotomy on endorachis and the extraction of right sympathetic ganglionated cord by means of sympathetic ganglionectomy.
Specimen : a) Posterior root in the region between a cut in posterior root and endorachis.
b) Posterior root in the region between spinal ganglion and endorachis.
c) Region right below spinal ganglion.

(5) A bilateral application of posterior rhizotomy on endorachis and the extraction of bilateral sympathetic ganglionated cord by means of sympathetic ganglionectomy.
Specimen : a) Posterior root in the region between a cut in posterior root and endorachis.
b) Posterior root in the region between spinal ganglion of posterior root and endorachis.
c) Region right below the spinal nerve ganglion.

(6) A bilaterel application of anterior and posterior rhizotomy in endorachis and the extraction of bilateral sympathetic ganglionated cord by means of sympathetic ganglionectomy.
Specimen : a) Posterior root in the region between a cut of posterior root and endorachis.
b) Posterior root in the region between spinal nerve ganglion and endorachis.
c) Region right below the posterior root of spinal nerve (peripheral)

Figure illustrates the dissection or extraction experiment schematically. Under a variety of conditions, specimens were collected on the 7th day after the date of operation from the dogs killed by heart-injection of Nembutal.

SECT. Ⅱ. METHOD OF OBSERVATION

The specimens were embedded in celloidine. Serial sections were prepared. As staining, modified method of BIELSCHOWSKY-SUZUKI was adapted to observe the structure of axis cylinder under microscope.

CHAP. Ⅲ. HISTOLOGICAL ASPECT OF SMALL SIZE NERVE FIBER IN THE NORMAL POSTERIOR ROOT.

Similarly to (Fig. 1) in which illustrates KOTERA's observation, our phase-difference microscopic observation about the nerve fiber with 1% Osmium stained myelinsheath (Fig. 2) reveals the small size myelinated nerve fiber with apparent Incisura of LANTERMANN and less stainable in comparison with large size myelinated nerve fibers. In (Fig. 3) represented by the stained axis cylinder by means of modified method of BIELSCHOWSKY-SUZUKI, we can notice extremely small size nerve fibers on its longitudinal-section which run in the wavy manner parallel to the large and medium size nerve fibers and hold consistency in width at least in the observed region.

In its cross-sectional area, groups of small size nerve fibers distribute among groups

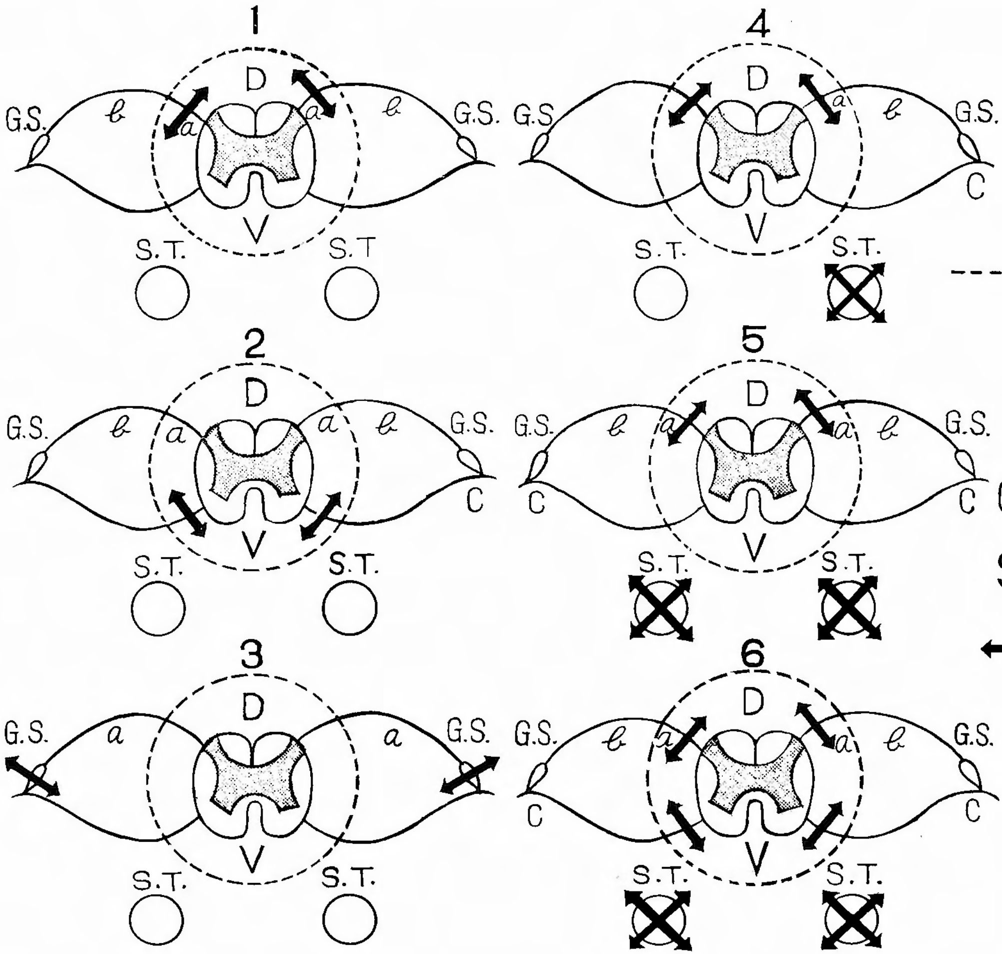

Figure illustrates the dissection or extraction experiment schematically

- - - - - Endorachis

D Posterior Root Region of Spinal Nerve

V Anterior Root Region of Spinal Nerve

G.S. Spinal Ganglion of Posterior Root

S.T. Sympathetic ganglionated Cord

⟷ Cut positions

of large and medium size nerve fibers and possesses heavily stained axis cylinder in the center portion being surrounded by the homocentric circular myelin sheath in common with the large and medium size nerve fibers.

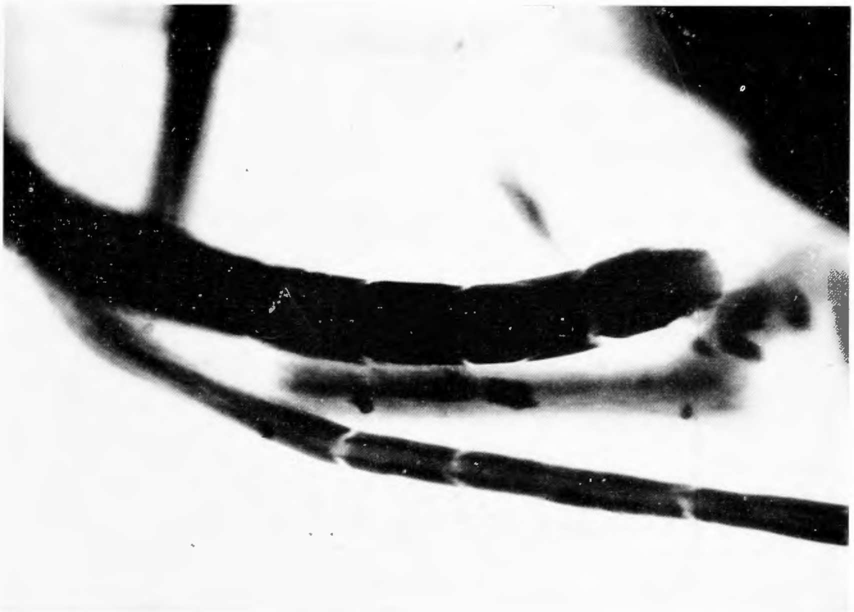

Fig. 1. Normal Posterior Root of Spinal Nerve (1% Osmium Stained) 600×

Fig. 2. Normal Posterior Root of Spinal Nerve (1% Osmium Stained) Observed under Phase Difference Microscope. 600×

Fig. 3. Normal Posterior Root of Spinal Nerve (Longitudinal Section, Axis Cylinder Staining) 400×

CHAP. IV. DEGENERATION EXPERIMENT.

PART I. RESULTS FROM POSTERIOR RHIZOTOMY (BILATERAL) IN ENDORACHIS.

In the central nerve side from cut face, large degree of degeneration became apparent in large and medium size nerves. In consequence, the formation of spinal bulb was observable in almost every fiber, also vacuole accumulation, feature of degeneration were obvious. As in (Fig. 4), remarkable increase of SCHWANN cells was also the case in each specimen so far investigated. However, the most part of small size nerve fiber showed no sign of degeneration, (Fig. 5) except that a few of them were not homogeniously stained, the axis cylinder of a few nerve fibers formed vacuole, (Fig. 6).

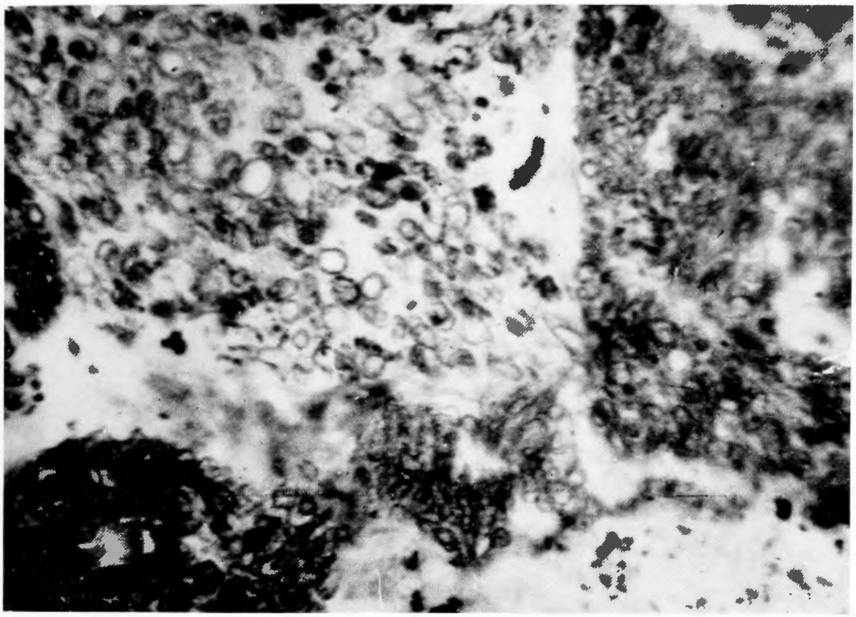

Fig. 4. Posterior Rhizotomy in Endorachis : Posterior Root in the Central Nerve Side from Cutting Edge. (Cross Section, Axis Cylinder Staining) 400×

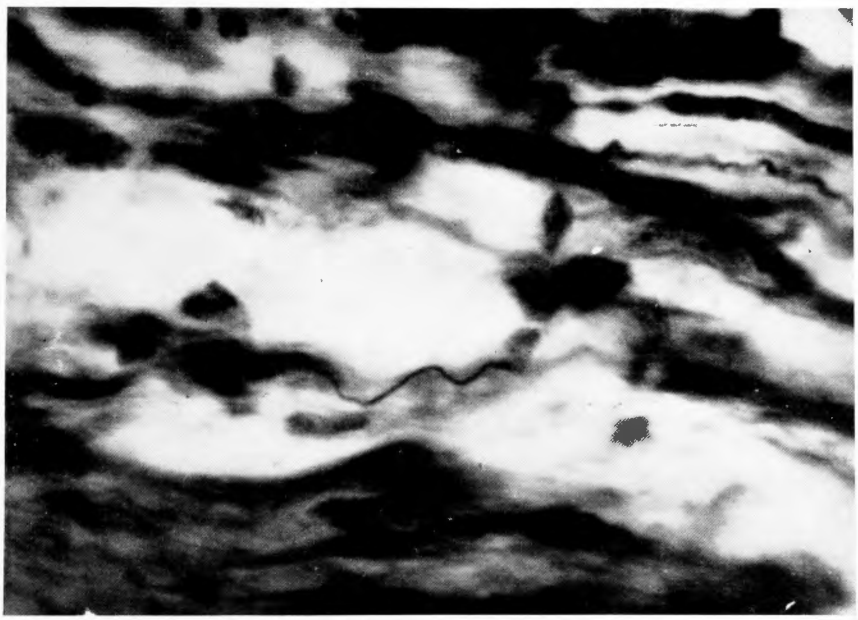

Fig. 5. Posterior Rhizotomy in Endorachis : Posterior Root in the Central Nerve Side from Cutting Edge. (Longitudinal Section, Axis Cylinder Staining) 600×

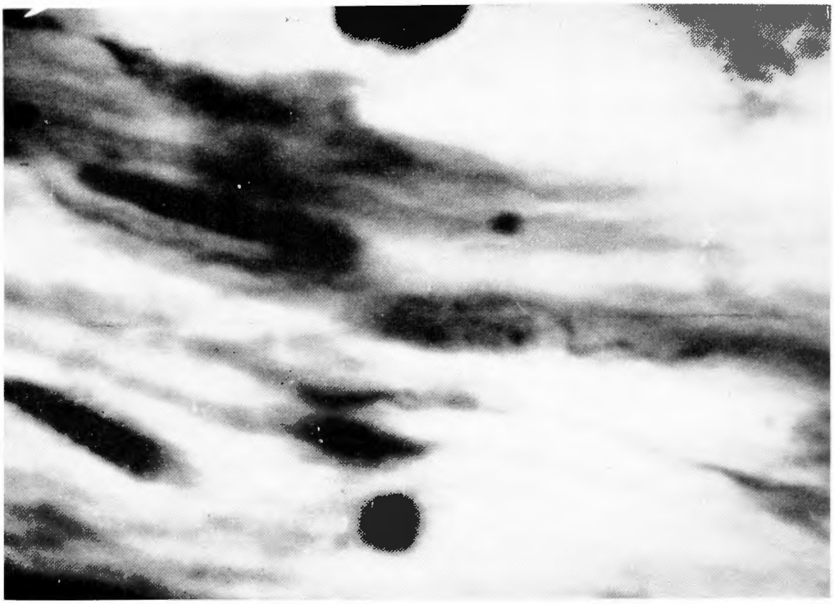

Fig. 6. Posterior Rhizotomy Endorachis : Posterior Root in the Central Nerve Side from Cutting Edge. (Longitudinal Section, Axis Cylinder Staining) 1,500×

In both regions between peripheral and a cut face, outside of endorachis and spinal-ganglion of posterior root, no sign of degeneration and the increase of SCHWANN cell was observed in the groups of large and medium size nerve fibers. The small size nerve fiber offered a intact looking as well. (Fig. 7, Fig. 8).

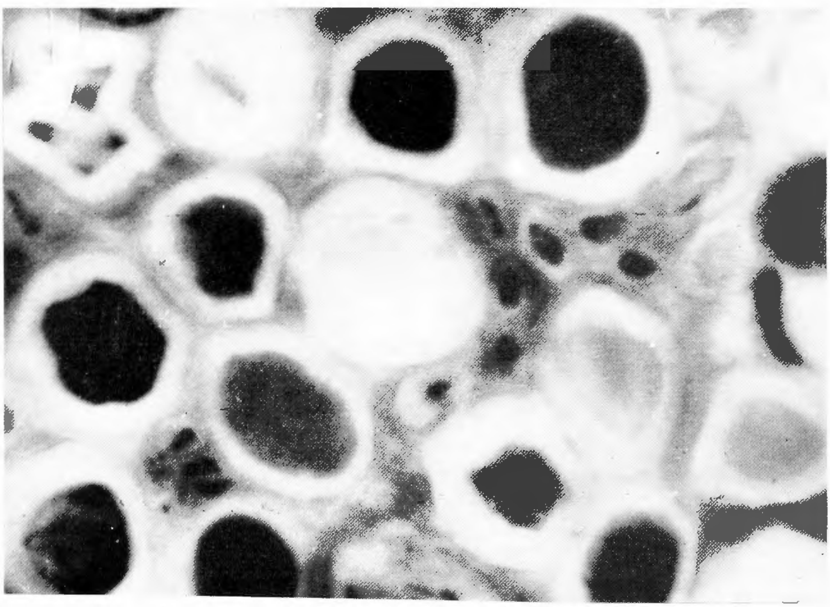

Fig. 7. Posterior Rhizotomy in Endorachis : Posteror Root in the Peripheral Region from Cutting Edge. (Cross Section Axis Cylinder Staining) 1,500×

Fig. 8. Posterior Rhizotomy in Endorachis : Posterior Root in the Peripheral Region from Cutting Edge. (Longitudinal Section, Axis Cylinder Staining) 600×

PART II. RESULTS FROM THE ANTERIOR RHIZOTOMY (BILATERAL) IN ENDORACHIS.

The results obstained in the central nerve side from endorachis indicated that the axis cylinders of large, medium and small size nerve fibers are in possession of good affinity against silver ion in the cross-sectional area. Fig. 9 illustrates the locality of small size nerve fiber group, three or four of which take place among the large and medium nerve fiber groups as samely as in the case of control experiment. Among the large, intermediary nerve fibers with intact axis cylinders, small size nerve fiber runs drawing an arc. (Fig. 10).

The results from the regions between endorachis and spinal ganglion make us comprehend that in the cross-section all types of nerve fiber are equally affinitive to silver ions and the small size nerve fiber locates as groups among large and medium size nerve fibers. (Fig. 11).

In the longitudinal section, the degree of staining of axis cylinder are exclusively equal, in addition, small size nerve fibers run drawing an arc among the large and medium size nerve fibers, being rich in silver affinity. (Fig. 12).

Results from the region right below the spinal ganglion of posterior root tell us the existence of large, medium size nerve fibers with axis cylinders of good silver affinity and no sign of degeneration or without axis cylinder and signs of degeneration in the form of vacuole accumulation.

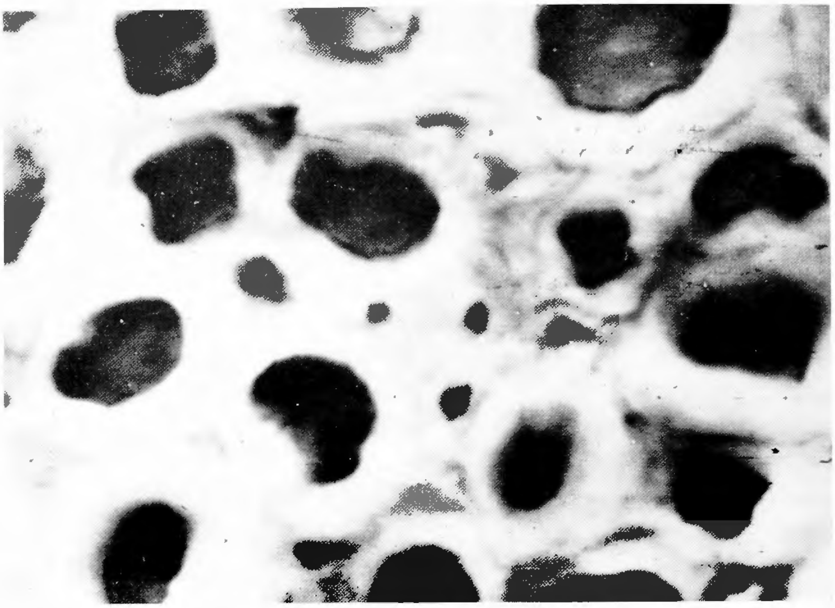

Fig. 9. Anterior Rhizotomy in Endorachis : Posterior Root in the Central Side from Endorachis (Cross Section, Axis Cylinder Staining) 1,500×

Fig. 10. Anterior Rhizotomy in Endorachis : Posterior Root in the Central Side from Endorachis (Longitudinal Section, Axis Cylinder Staining) 1,500×

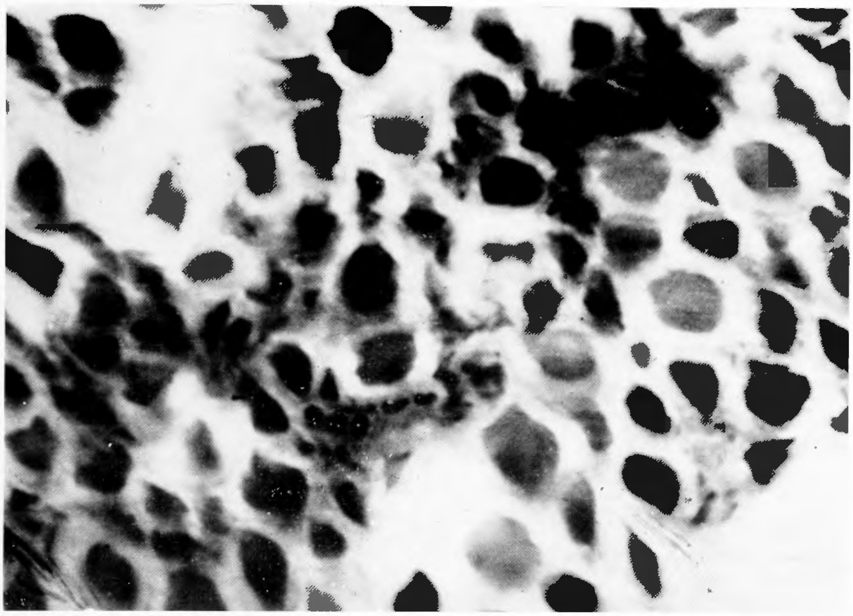

Fig. 11. Anterior Rhizotomy in Endorachis : Posterior Root in the Region between Posterior Root Nerve Ganglion and Endorachis. (Cross Section, Axis Cylinder Staining) 600×

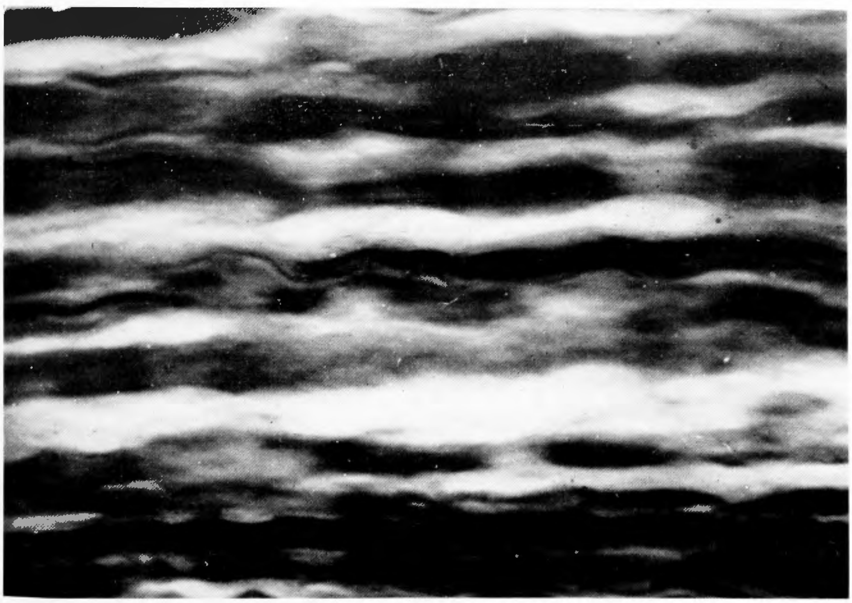

Fig. 12. Anterior Rhizotomy in Endorachis : Posterior Root in the Region between Posterior Root Nerve Ganglion and Endorachis (Longitudinal Section, Axis Cylinder Staining) 600×

The diameter of vacuole resembles to the width of nerve fiber. The latter can only be distinguished from the neighboring nerve fibers by the trace of SCHWANN cells. However, the small size nerve fibers as similary located in a groups of 3 or 4 fiber units as it is seen in the normal fibers, possesses the axis cylinder of good silver affinity. (Fig. 13).

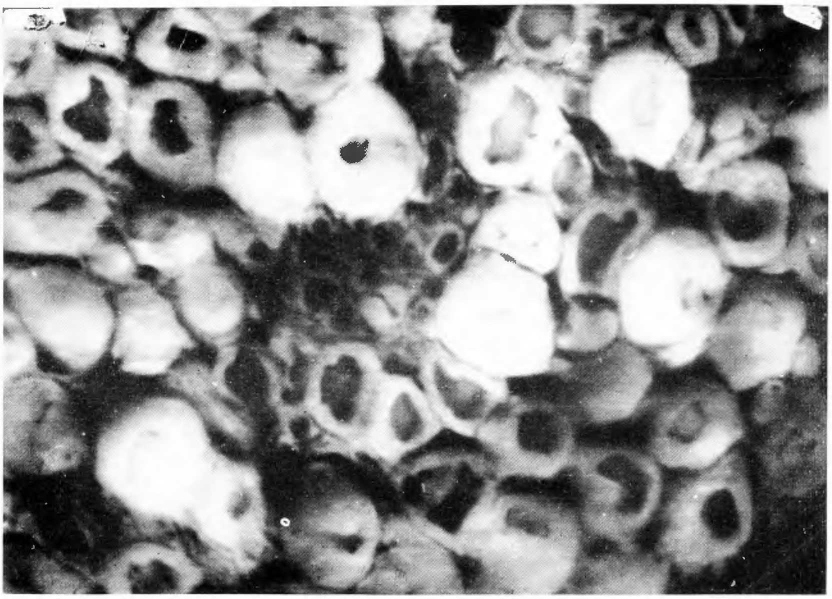

Fig. 13. Anterior Rhizotomy in Endorachis : The Region right below the Nerve Ganglion of Posterior Root. (Cross Section, Axis Cylinder Staining) 600 ×

Fig. 14. Anterior Rhizotomy in Endorachis : The Region right below the Nerve Ganglion of Posterior Root. (Longitudinal Section, Axis Cylinder Staining) 1,500 ×

In the longitudinal section, small size nerve fiber runs smoothly drawing an arc among the medium and large nerve fibers, in which axis cylinder is less stainable than that in large size nerve fiber.

On the above course, disintegration of axis cylinder, accumulation of vacuole, swelling as a token of degeneration were not observed. (Fig. 14).

PART Ⅲ. RESULTS FROM THE POSTERIOR RHIZOTOMY (CENTRAL)

In large, medium nerve fibers, as revealed by means of the rhizotomy in the central side of a cut face of posterior root, typical feature of degeneration appeared in spinal bulb, formation, vacuole accumulation in axis cylinder, the increase of SCHWANN cells.

In small size nerve fiber except a narrow area, cylinder stains densely and partly disintegrates into segments and swells. (Fig. 15, 16, 17, 18).

Nevertheless, many of the small size nerve fibers do not seem to differ significantly from the normal posterior root with respect to drawing arc, degree of staining, and its size. (Fig. 19).

Fig. 15. Central Rhizotomy of Nerve Ganglion of Posterior Root : Posterior Root in the Central Nerve Side from Cutting Edge. (Longitudinal Section, Axis Cylinder Staining) 600×

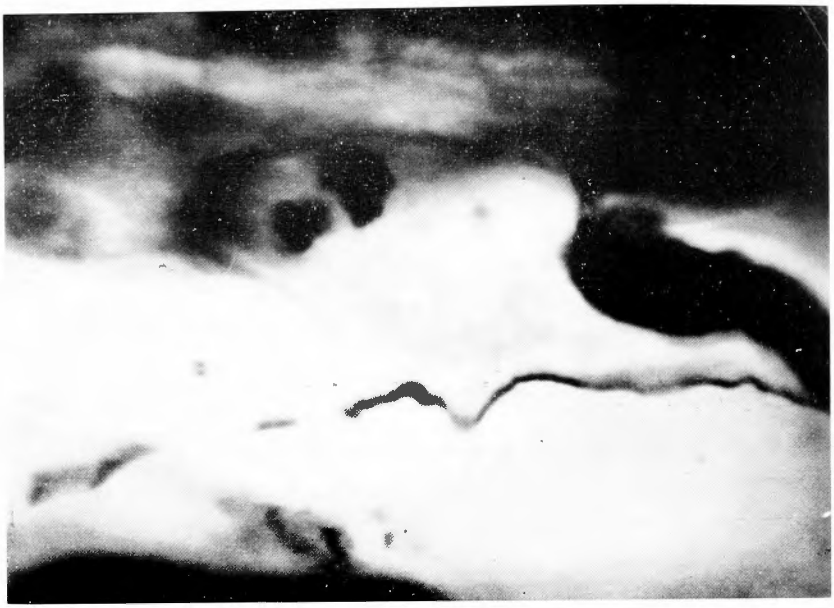

Fig. 16. Partial Magnification of Fig. 15 Picture (Axis Cylinder Staining) 1,500 ×

Fig. 17. Central Rhizotomy of Nerve Ganglion of Posterior Root: Posterior Root in the Central Nerve Side from Cutting Edge. (Longitudinal Section, Axis Cylinder Staining) 1,500 ×

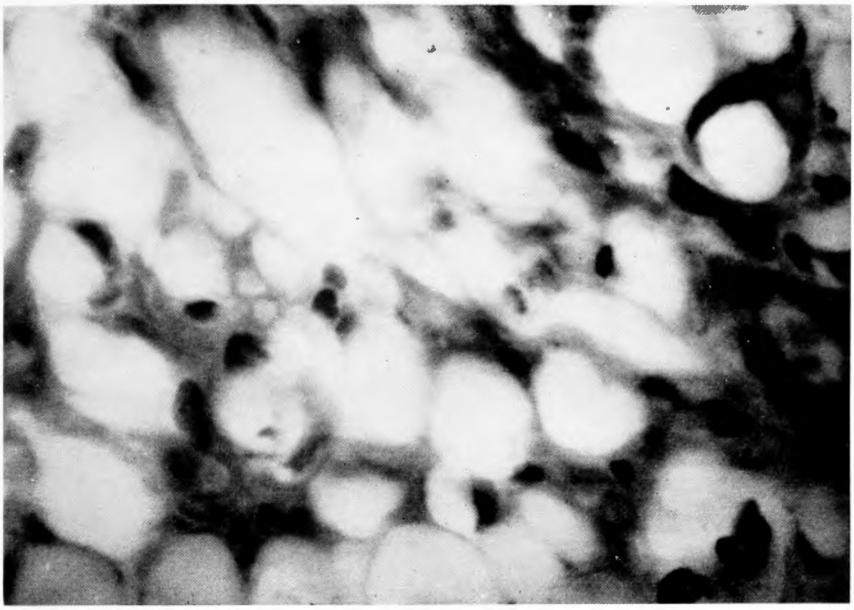

Fig. 18. Centrnl Rhizotomy of Nerve Ganglion of Posterior Root : Posterior Root in the Central Side from Cutting Edge. (Cross Section, Axis Cylinder Staining) 600×

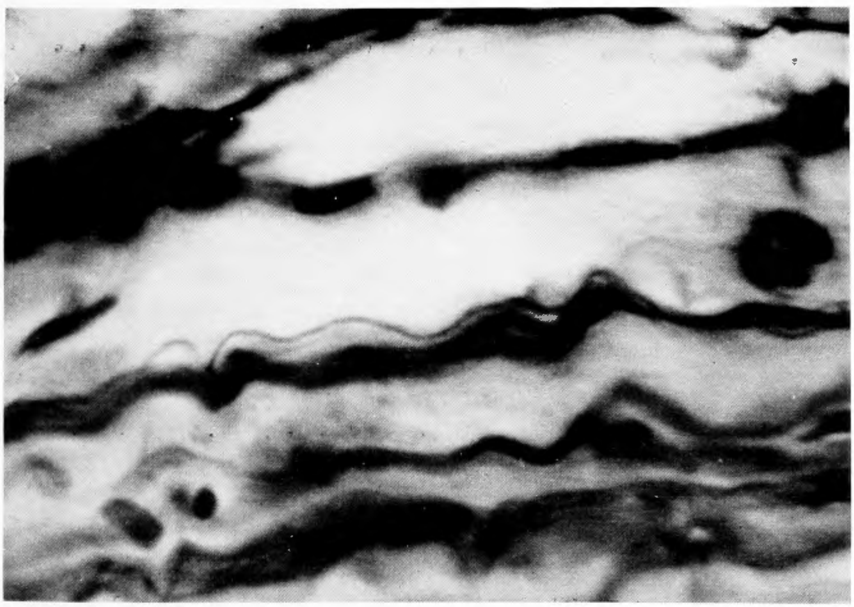

Fig. 19. Central Rhizotomy of Nerve Ganglion of Posterior Root : Posterior Root in the Central Nerve Side from Cutting Edge. (Longitudinal Section, Axis Cylinder staining) 600×

PART IV. RESULTS FROM THE POSTERIOR RHIZOTOMY (BILATERAL) OF ENDORACHIS AND EXTRACTION OF RIGHT SYMPATHETIC GANGLION.

In the region between cut face of posterior root and endorachis, nearly half numbers of large, medium nerve fibers demonstrated the spinal bulb formation, vacuole accumulation in axis cylinder, disintegration, collapse, whereas the small size nerve fiber stayed normal with respect to silver affinity, locality, and size. (Fig. 20, 21).

In the region between spinal ganglion of posterior root and endorachis, the sign of degeneration was approved in large and medium size nerve fibers with overall good silver affinity and homogeneity. In the cross-section, small size nerve fiber is rich in silver affinity as a whole, and runs in a wavy manner as in the normal. (Fig. 22, 23).

No trace of degeneration was found in large and medium size nerve fibers in the region right below the spinal ganglion of posterior root, being rich in silver affinity.

As shown in Fig. 24, 25, the small size nerve fiber possessing good silver affinity and running in a flat wavy manner is located among the large and medium size nerve fibers and obviously display the axis cylinder surrounded by concentric circular myelinsheath.

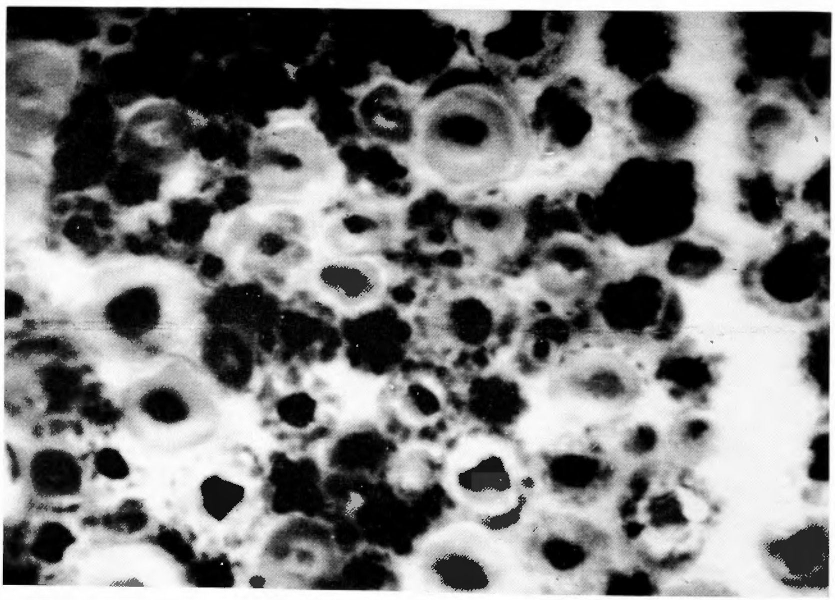

Fig. 20. Posterior Rhizotomy in Endorachis (Bilateral) and Right Sympathetic Ganglionectomy : In the Posterior Root Region between Endorachis and Cutting Edge. (Cross Section, Axis Cylinder Staining) 600×

Fig. 21. Posterior Rhizotomy in Endorachis (Bilateral) and Right Sympathetic Ganglionectomy : In the Posterior Root Region between Endorachis and Cutting Edge. (Longitudinal Section, Axis Cylinder Staining) 600×

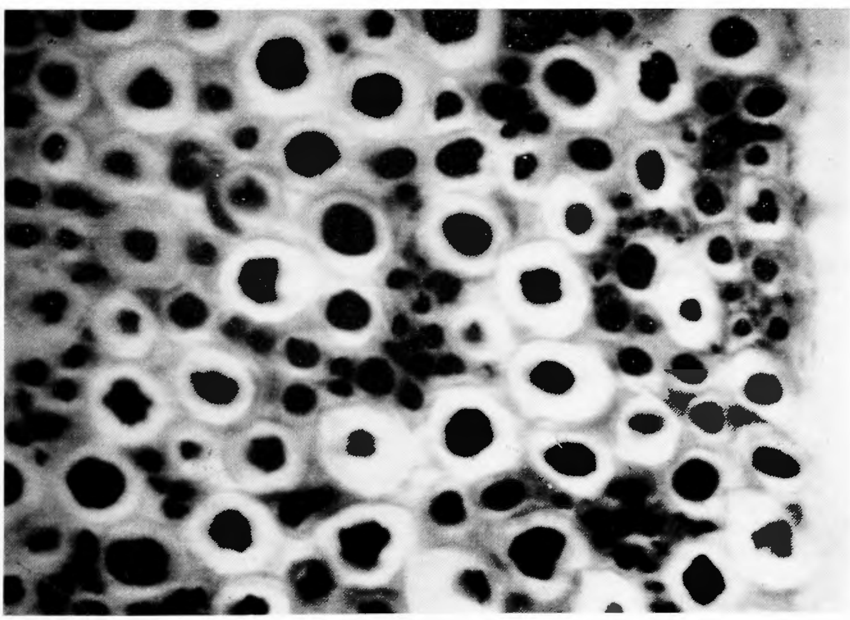

Fig. 22. Posterior Rhizotomy in Endorachis (Bilateral) and Right Sympathetic Ganglionectomy : In the Region between Endorachi and the Nerve Ganglion of Posterior Root. (Cross Section, Axis Cylindder Staining) 600×

Fig. 23. Posterior Rhizotomy in Endorachis (Bilateral) and Right Sympathetic Ganglionectomy : In the Region between Endorachis and the Nerve Ganglion of Posterior Root. (Longitudinal Section, Axis Cylinder Staining) 600×

Fig. 24. Posterior Rhizotomy in Endorachis (Bilateral) and Right Sympathetic Ganglionectomy : The Region right below the Nerve Ganglion of Posterior Root. (Longitudinal Section, Axis Cylinder Staining) 600×

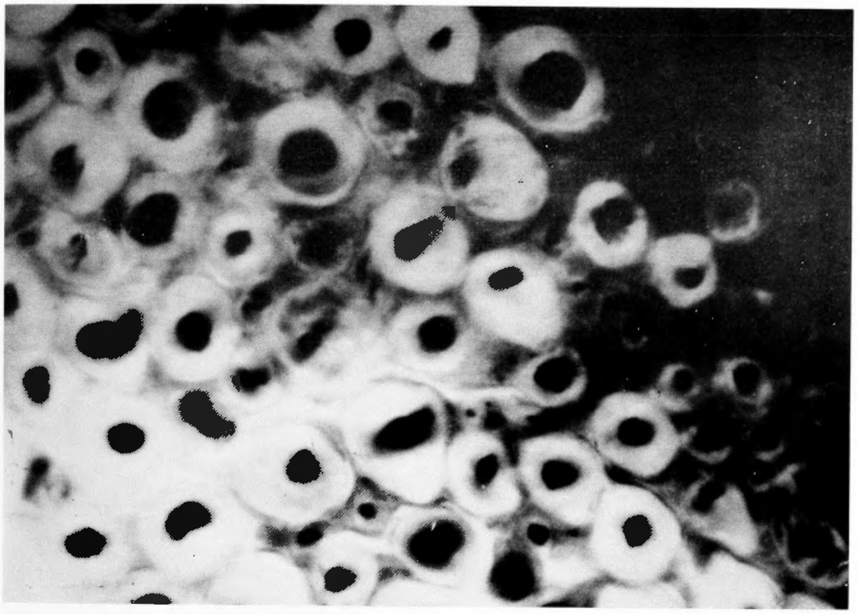

Fig. 25. Pestorior Rhizotomy in Endorachis (Bilateral) and Right Sympathetic Ganglionectomy : The Region right below the Nerve Ganglion of Posterior Root. (Cross Section, Axis Cylinder Staining) 600×

PART V. RESULTS FROM THE POSTERIOR RHIZOTOMY (BILATERAL) OF ENDORACHIS AND THE EXTRACTION OF BILATERAL SYMPATHETIC GANGLIONS

In the posterior root region between the cut face and endorachis almost half numbers of large and medium size nerve fibers in the cross-section failed to retain their axis cylinder and lowered the affinity to silver ion, which was seemingly alike the results from Part IV. However, small size nerve fiber which take place in groups of 2 or 3 units fibers among well degenerated large and medium nerve fibers, was largely resistant to degeneration and kept intact as being represented by Fig. 26.

In the region between endorachis and spinal ganglion of posterior root, large and medium size nerve fibers were found no indication of being degenerated, also small size nerve fiber possessed rich affinity to silver ion and its arrangement followed the smooth wavy manner without any token of degeneration. (Fig. 27, 28)

In the region right below the spinal ganglion of posterior root, large and medium size nerve fibers retain the axis cylinder of good silver affinity and do not include the devoid of axis cylinder or the inhomogeniously stained structure. In the cross section, homogenious staining and other normal features were the actual case. In the cross-section of small size nerve fiber, these can be counted in groups of 2 or 3 units fibers of normal feature among large and medium nerve fibers. In these longitudinal sections, possessing homogenious affinity to silver ion and arranging in a smooth wavy manner, no features of degeneration such as disintegration, collapse, and affinity to stains were not demonstrated. (Fig. 29, 30)

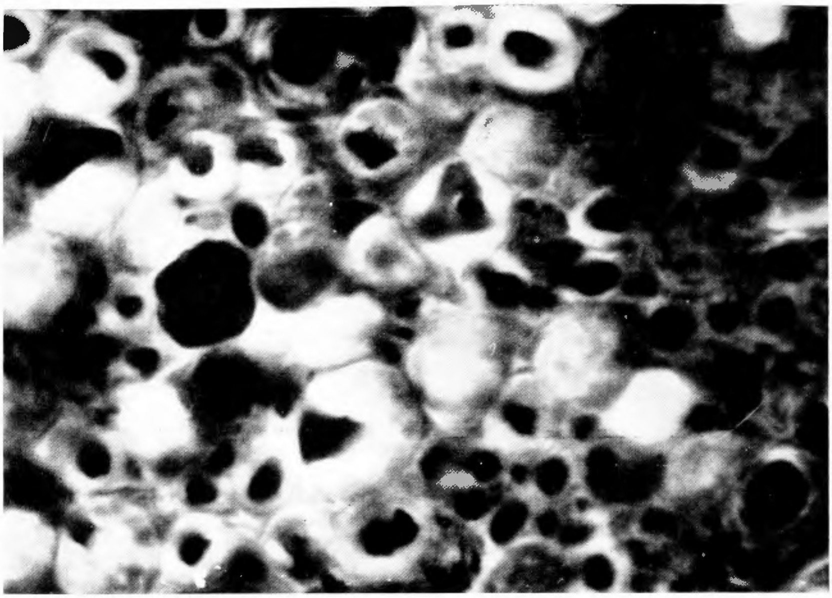

Fig. 26. Posterior Rhizotomy in Endorachis (Bilatrral) and Bilateral Sympathetic Ganglionectomy : In the Posterior Root Region between Endorachis and Cutting Edge. (Cross section, Axis Cylinder Staining) 600×

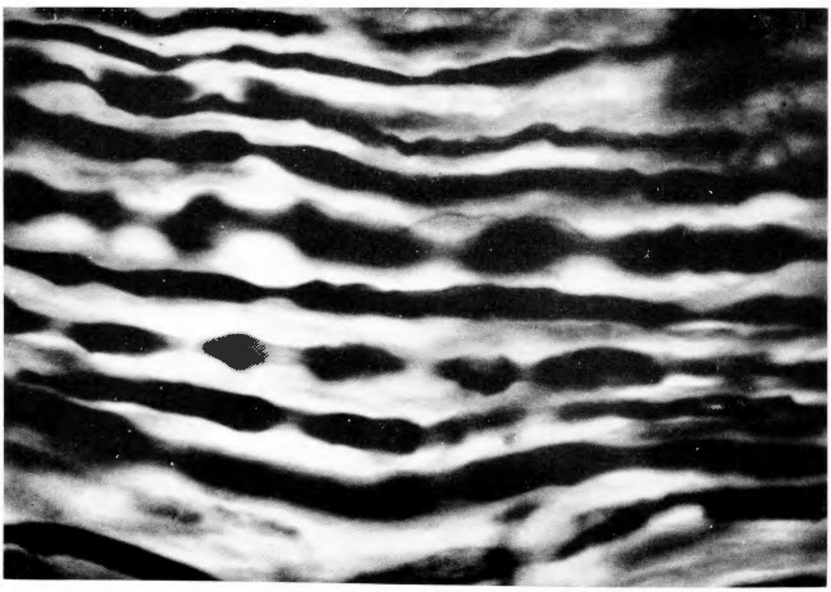

Fig. 27. Posterior Rhizotomy in Endorachis (Bilateral) and Bilateral Sympathetic Ganglionectomy : In the Posterior Root Region between Endorachis and Cutting Edge. (Longitudinal Section, Axis Cylinder Staining) 600×

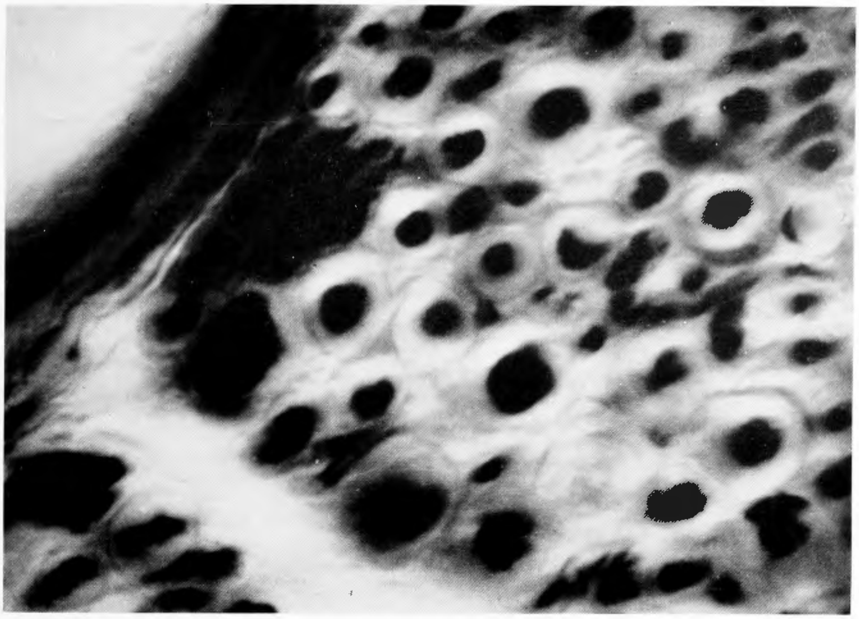

Fig. 28. Posterior Rhizotomy in Endorachis (Bilateral) and Bilateral Sympathetic Ganglionectomy . In the Region between Cutting Edge and Nerve Ganglion of Posterior Root. (Cross Section, Axis Cylinder Staining) 600 ×

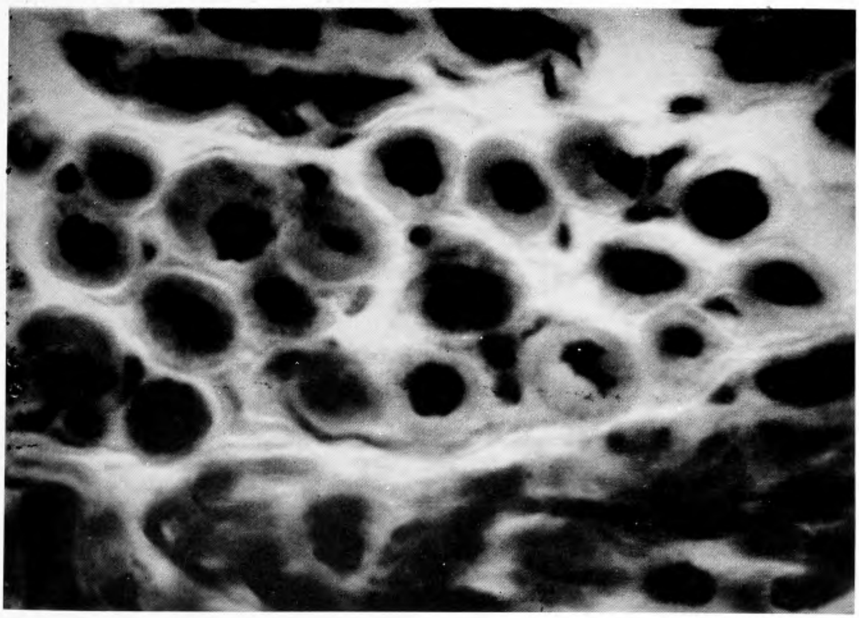

Fig. 29. Posterior Rhizotomy in Endorachis (Bilateral) and Bilateral Sympathetic Ganglionectomy : The Region right below the Nerve Ganglion of Posterior Root. (Cross Section, Axis Cyliner Staining) 600 ×

Fig. 30. Posterior Rhizotomy in Endorachis (Bilateral) and Bilatoral Sympathetic Ganglionectomy : The Region right below the Nerve Ganglion of Posterior Root. (Longitudinal Section, Axis Cylinder Staining) 600 ×

PART VI. RESULTS FROM THE POSTERIOR AND ANTERIOR RHIZOTOMY AND THE EXTRACTION OF BILATERAL SYMPATHETIC GANGLIA.

In the posterior region between endorachis and cut face, almost half numbers of large and medium size nerve fiber revealed the lowering of silver affinity, the partial disintegration and collapse of axis cylinder which is the typical characteristics of degeneration. Contrarily, small size nerve fiber retained the previously described normal features. (Fig. 31).

In the region between endorachis and spinal ganglion of posterior root, large and medium size nerve fibers exhibited the axis cylinder of good silver affinity in cross-sectional area. In the longitudinal section, large and medium size nerve fibers are obviously shown and its silver affinity is also homogenious. Small size nerve fiber, in cross-sectional area, distributes in groups of 2 or 3 units fibers among the groups of medium size nerve fibers and its axis cylinder also possesses the affinity to silver ions. Besides, in longitudunal section, no sign of degeneration were demonstrated in small size nerve fibers as shown in Fig. 32, 33.

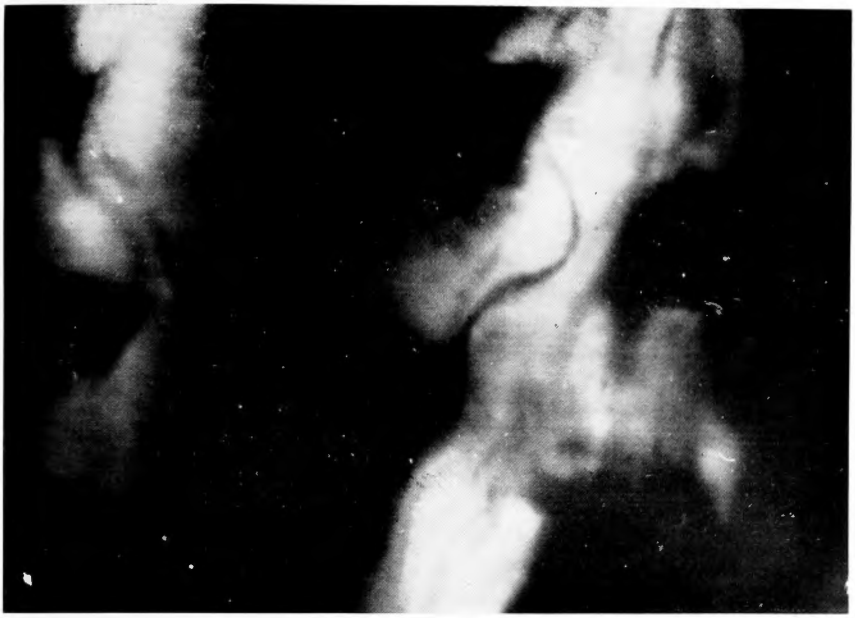

Fig. 31. Anterior and Posterior Rhizotomy (Bilateral) and Bilateral Sympathetic Ganglionectomy : In the Posterior Root Region between Cutting Edge and Endorachis. (Longitudinal Section, Axis Cylinder Staining) 1,500 ×

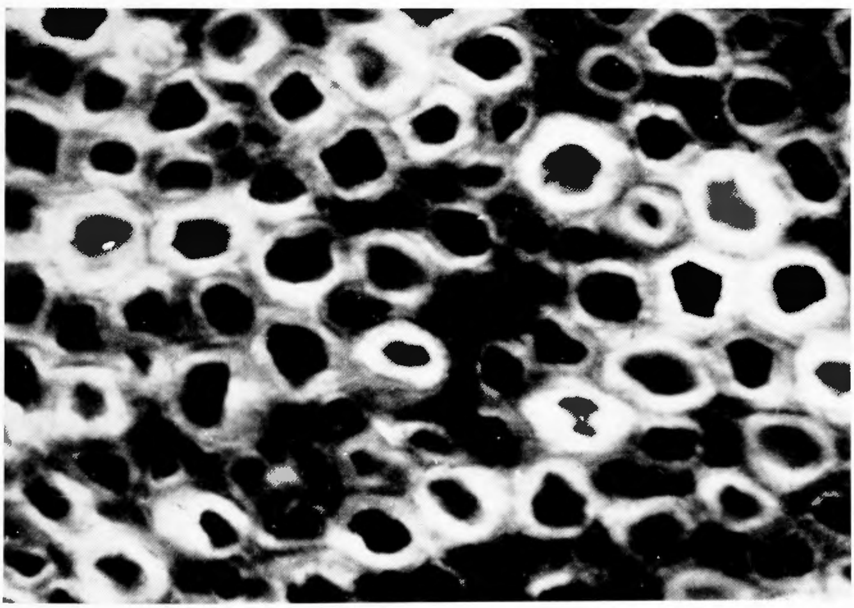

Fig. 32. Anterior and Posterior Rhizotomy (Bilateral) and Bilateral Sympathetic Ganglionectomy : In the Posterior Root Region between Endorachis and Nerve Ganglion of Posterior Root. (Cross Section, Axis Cylinder Staining) 600 ×

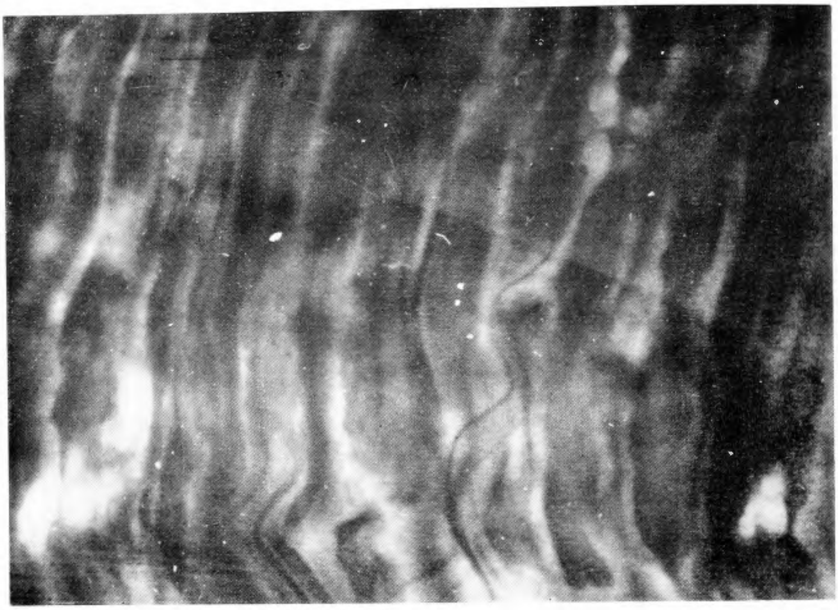

Fig. 33. Anterior and Posterior Rhizotomy (Bilateral) and Bilateral Sympathetic Ganglionectomy : In the Posterior Root Region between Endorachis and Nerve Ganglion of Posterior Root. (Longitudinal Section, Axis Cylinder Staining) 600×

In the region right below the spinal ganglion of posterior root, in the cross-sectional area, all types of nerve fibers so far mentioned revealed the features of degeneration in a varied degree. In cross-section, almost all of the axis cylinder of large and medium size nerve fibers disappeared.

In the cross-section of small size nerve fibers, axis cylinder also disappeared and accompanies only a trace of myelinsheath on its surroundings so that these features do not make small size nerve fibers appear as they were before. Consequently, in above specimen, all types of nerve fibers demonstrate complete disintegration, collapse, and disappearance as being represented by Fig. 34, 35.

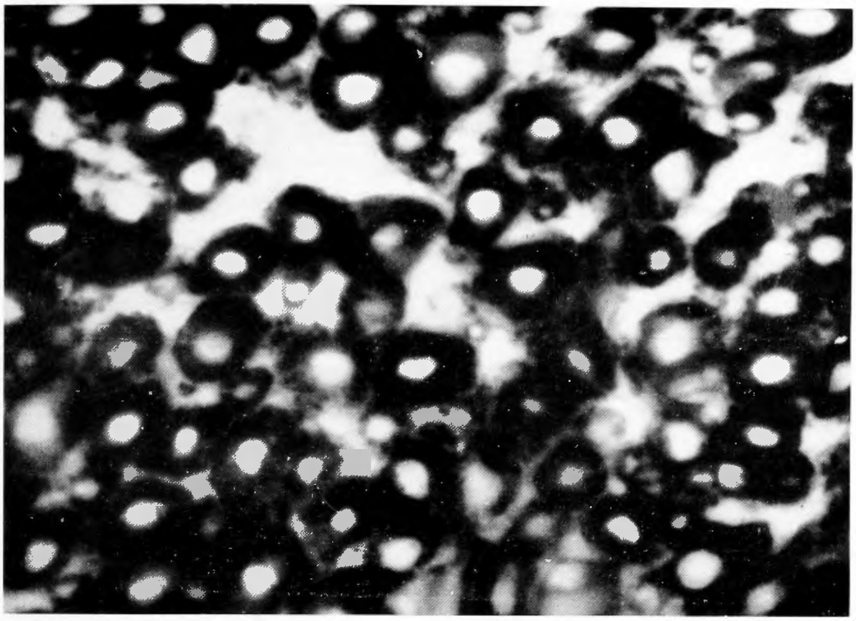

Fig. 34. Anterior and Posterior Rhizotomy (Bilateral) and Bilateral Sympathethic Ganglionectomy : The Region right below the Nerve Gamglion of Posterior Root. (Cross Section, Axis Cylinder Staining) 600×

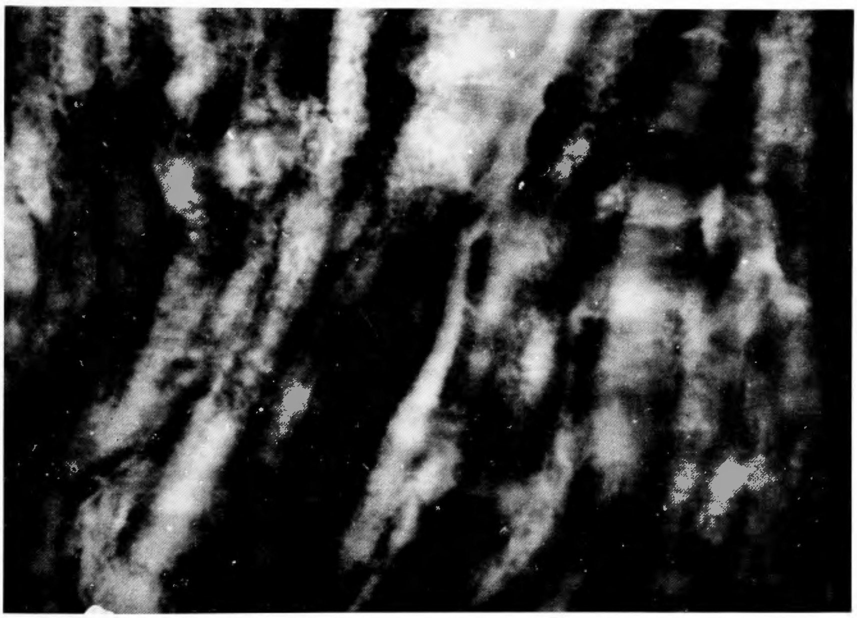

Fig. 35. Anterior and Posterior Rhizotomy (Bilateral) and Bilateral Sympathetic Ganglionectomy : The Region right below the Nerve Ganglion of Posterior Root. (Longitudinal Section, Axis Cylinder Staining) 600×

CHAP. V. DISCUSSION AND SUMMARY

In the normal posterior root of spinal nerve, the observation of 1% Osmium stained specimen under the phase-difference microscopy enabled us to realize the presence of small size myelinated autonomic nerve fibers.

Our observation is in line with works of DIAMAREA de MENNATO (1920) with polarized light and the GASSER'S observation (1955) under electron microscope which led them to deny the basis of distinguishing myelinated nerve fibers from the unmyelinated.

Our observation supports Osmium staining method of discernment between spinal nerve and autonomic nerve by NAKANISH, and the recent view of KOTERA (1959) as to autonomic nerve, being myelinated. In the occurrence of axis cylinder staining by modified method of BIELSCHOWSKY-SUZUKI, the normal features were to observe the autonomic nerve fiber running through the space among the groups of nerve fiber in a smooth wavy manner.

In the cross section, we visualized the groups of small size nerve fibers positioning among large and medium nerve fiber groups. The fact that small size myelinated nerve fibers remain intact in spite of the marked appearance of WALLER degeneration among sensory nerve fiber groups in the side of spinal nerve from a cut face signify obviously the existence of autonomic nerve fiber which begins at spinal cord and runs centrifugally along the posterior root of spinal nerve.

This fact also confirms GO's observation that the cutting of posterior root did not cause degeneration on myelinated nerve fibers in the central nerve side. NAKANISHI supported above observation as well.

On the other hand, in the peripheral side of dissected posterior root from endorachis, no trace of degeneration was discovered in the autonomic nerve fibers.

In 1889, GASKELL evidenced the presence of unmyelinated nerve fibers which enters into the posterior root of spinal nerve through grey rami communicantes, beginning at ganglionated cord of dogs. RANSON in 1911, WILSON in 1920, also approved in dogs that unmyelinated nerve fibers could be traced until in spinal cord through ganglion of spinal nerve and posterior root. In 1924, Müller recognized the unmyelinated nerve fiber running to the central nerve from grey matter, though not being clarified the pathway through posterior root and entrance into spinal cord. YAMAZAKI (1926) observed the presence of unmyelinated nerve fibers in each part of posterior root which could be followed to spinal nerve. As it has been known that the centripedal autonomic nerve fibers exist in the posterior root of spinal cord.

From the dissection experiments, the above also became true. The results from posterior rhizotomy (central) indicated that in the central side of autonomic nerve fiber with swelled axis cylinder disintegration, accumulation of vacuoles which is the known characteristics of WALLER degeneration were noticed. The findings that many normal autonomic nerve fibers remained besides the nerve fibers suffered from WALLER degeneration lead us to deduce the existence of two types of nerve fibers in posterior root of spinal nerve, namely, centripetal and centrifugal nerve fibers.

However, no features of degeneration were shown in the small size nerve fibers right below the posterior root of spinal ganglion at the time of anterior rhizotomy. This means that the autonomic nerve fiber in the posterior root of spinal nerve is not effected after an application of anterior rhizotomy. Hence no fibers can be thought of entering into posterior root through the anterior and posterior roots of spinal nerve.

From the anterior rhizotomy, the coexistence of normal and denatured features in large and medium nerve fibers became apparent, which inevitably correspond to the cooperation between sensory nerve system and motor nerve system.

In 1842, BIDDER and VOLKMANN confirmed that at the path far from sympathetic ganglionated cord, namely, at lumbar region of frog, the small size myelinated nerve fibers enter into spinal nerve and direct towards peripheral region. From his anatomical, physiological view points on the nervous control of blood vessel, MÜLLER was suggestive of that against peripheral vessel autonomic nerve fibers reach the sympathetic nerve by way of 8th cervical region of spinal cord and the central nerve of the 3rd lumber region of spinal cord and anteriorroot in orderly fashion, and thus the nerve fibers extended to mingled spinal nerves runs parallel to the spinal nerve.

While BAYLISS insists the 11th dorsal or the 3rd lumbar region of the spinal nerve being the position from where fibers go through anterior root and grey rami communicantes and enter into sympathetic nerve ganglion. LANGLEY, on the other hand, proposed the 6th lumbar and the 2nd sacral region of spinal nerve as the responsible places, WERZILOFF considered that in the height between 4th lumbar and the 1st sacral region of spinal nerve, the blood vessel controlling nerve fibers spread over the peripheral region, accompanying autonomic nerve fibers by way of grey rami communicantes from sympathetic nerve ganglion.

In 1950, NAKANISHI intended to search for the mingled state of fibers morphologically characterized by sympathetic nerve fibers in the rest component exclusive of the fibers originated in spinal nerve fibers, and finally attained the conclusive remarks that there were no autonomic nerve fibers which begins simultaniously at spinal nerve ganglion with spinal nerve fiber. According to the above observation, in the peripheral region none of autonomic nerve fiber was traced as long as the sympathetic ganglionated cord is removed as much as possible.

However, from our sympathetic ganglionectomy of one side and bilateral posterior rhizotomy, no remarks of degeneration were found in the posterior root of autonomic nerve fiber. Also, in the region right below the posterior root of nerve ganglion, only the autonomic nerve fiber with normal features was found. In the sympathetic ganglionectomy and bilateral posterior rhizotomy as well, the normal autonomic nerve fiber would be observed in the central nerve side of the posterior root of spinal nerve. By the reason that the dissection experiment is not in concern with the posterior root in autonomic nerve fiber, we can assume the process in which involves the common path with sensory spinal nerve extending to posterior root.

Finally we made a trial to dissect the bilateral sympathetic ganglionated cord and bilateral anterior and posterior roots with the purpose of blocking the central path which seems to be introduced into the posterior root of spinal nerve and peripheral nerve trunks.

Consequently, we noticed autonomic nerve fibers with normal features in the posterior region between the posterior root of nerve ganglion and endorachis and inside of endorachis, whereas in the region below ganglion of posterior root axis cylinder of large and medium size nerve fibers and all of the autonomic nerve fibers as well accompanied the large-scale degeneration. Posterior root of spinal nerve ganglion, having been simply acknowledged as the cell station in the sensory nerve fibers of spinal nerve involve a few types of cells, namely, one with cholinesterase activity, monoaminoxidase activity, positive staining type of Nucleus, which OKINAKA clarified by histochemical observation in 1960.

OKINAKA's evidence is not sufficient to fullfil all the requirements to choose one from two prevailing theories, so-called Inverse Theory of NERVE CONDUCTANCE and THEORY of Para-sympathetic Spinal Nerve Existence, but sufficient to provide some biological importance in the future decade. Being obvious from our experiments, blocking of all the path directed to periphery, causes the complete degeneration of nerve fibers.

When leaving posterior root of nerve ganglion intact, the groups of nerve fiber with their origin in posterior root of nerve ganglion keep alive. As a matter of conjecture, posterior root of spinal ganglion is not simply a cell station of sentosory nerve fiber, but the histochemically proved center of nutrition of biological importance. In 1957, SETO raised a problem about vegetative terminal reticulum, which did not come from free endings, but formed networks with differently originated branches. Hence, the endings of vegetative nerve system are in the form of huge nervous network and even in the basal portion the same pattern can be recognized. Probably, the whole system is in the closed form of network structure. Again, it would seem that the autonomic nerve fiber in the posterior root of spinal nerve can not be distinguished from the rest without the complete disruption of spinal nerve of posterior root.

CHAP VI. CONCLUSIVE REMARKS.

With the purpose of attempt to search for the origin of autonomic nerve fiber in the posterior root of spinal nerve, we undertook the dissection experiments with adult dogs weighing 10kg under a variety of conditions. The degree of degeneration of nerve fibers as indices led us to conclude as follows :

1) Small size, myelinated autonomic nerve fiber distributes in the posterior root of spinal nerve.
2) Autonomic nerve fibers in the spinal posterior root include two types, that is, of centripetal and centrifugal properties.
3) The anterior rhizotomy of spinal nerve did not effect on the feature of autonomic nerve fiber in posterior root. The region right below the posterior root of spinal ganglion consists of the groups of sensory nerve fibers and motor nerve fibers. These two groups exist as the mingled bundle and in the inseparable state.
4) The rhizotomy at the central portion of the posterior root of spinal ganglion could cause WALLER degeneration in the part of autonomic nerve fibers.
5) The removement of 1 or bilateral sympathetic ganglionted cord did not effect significantly on the autonomic nerve fiber in posterior root.
6) The blocking of the majority of nerve path after the removement of anterior and posterior roots of spinal nerve and bilateral sympathetic nerve ganglion,

remained many of the nerve fiber in the posterior root normal, while in the region right below the posterior root of nerve ganglion, the large, medium, and small size nerve fibers revealed the features of degeneration in the majority.

7) Though the posterior root of spinal nerve is known to be a cell station of sensory nerve path, it would seem that autonomic nerve fibers does not only take a part as cell station, but also plays a role as the center of nutrition and biological importance.

Auther wishes to express his sincere gratitude to prof. Dr. EISHI KONDO for the preparation of manuscript and his encouragement throughout this experiment. Auther also indebted to Dr. TOSHIHARU KOTERA fo his kind help in many ways of doing experiments.

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和文抄録

脊髄神経後根に於ける自律神経線維の由来に関する実験的研究

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巽　典三

脊髄神経の後根束に於ける自律神経線維の存在とその本態に関しては，生理学的にも薬理学的にも種々の研究がなされているが，他方解剖学的にも亦幾多の先人の研究業績がある．即ち第一に血管支配神経の解剖学的見地によると，血管拡張神経と知覚神経とは，その神経線維の同一性は別問題として，両者は極めて密接な関係にあるといわれ，又脊髄神経後根中に血管拡張神経の存在が実験的に認められている．

第二に組織学的見地に従えば，脊髄神経後根中に小径の無髄線維の存在が認められて以来，現在では小径有髄性の自律神経線維が存在する事が確認せられている．

第三に組織化学的見地より，自律神経線維をCholine作動性神経，Adrenaline作動性神経に大別するという理論が提唱されている．

然しながら血管支配神経の解剖学的見地よりしても，又組織学的見地よりしても，はた又組織化学的見地よりの何れの見地よりしても，脊髄神経後根中に於ける自律神経線維の走行経過並びにその由来に関してはまだ解明されてはいない．

従つて私は約10kgの成犬を実験材料として脊髄神経中に於ける自律神経線維の存在を追求し，Osmium酸の短時間染色標本の位相差顕微鏡所見並びにBielschowsky氏染色法鈴木氏変法による染色標本に於て之を確認し，次いで脊髄神経後根中に於ける自律神経線維の由来を追求するために各種切断実験を行ない，之等より採取した神経を，Bielschowsky氏染色法鈴木氏変法を用いて観察した結果次の所見を得た．

(1) 脊髄神経後根中に小径有髄の自律神経線維を確認した．

(2) 脊髄神経後根中の自律神経線維には，遠心性のものと求心性のとの2種類が存在する．

(3) 脊髄神組前根切断実験では，後根中の自律神経線維に何等の影響を与える事は出来ない．

(4) 脊髄後根神経節の中央部切断により後根中の自律神経線維の一部に変性を惹起せしめ得る．

(5) 一側並びに両側交感神経節節状索を剔除しても後根中の自律神経線維には殆んど認むべき影響を与える事は出来ない．

(6) 脊髄前根，後根並びに両側交感神経節状索を剔除し殆んどすべての神経路を遮断しても尚後根中には多くの正常自律神経線維を認める事が出来る．然しこの場合には脊髄後根神経節直下部に於ては，あらゆる神経線維は高度の変性，崩壊を来している事が認められる．

以上の実験の結果，後根内の自律神経線維を変性せしめ得たものは，脊髄神経後根神経節中央部での切断実験のみである事を知つた．

この事は脊髄後根中の自律神経線維に関して，脊髄後根神経節が極めて重大な意義を有するものである事を意味するものである．

脊髄後根神経節は知覚神経路のCell Stationであると言われるが，事自律神経線維に関しては，単純なCell Stationに止まらず，それ以上の重要な意義のある部位であると考えられる．即ち自律神経本来の，所謂生命神経としての，生命の根源をなす栄養中枢的役割を果しているものと推測し得るのである．